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# COMPARATIVE IMMUNOHISTOCHEMICAL ASSESSMENT OF THE EFFECTS OF CHITOSAN AND CURCUMIN ON DORSAL SURFACE OF TONGUE MUCOSA OF ALBINO RATS FED ON HIGH-FAT DIET: HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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***KEYWORDS***

*High-fat diet, Curcumin, Chitosan, Tongue.*

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## *ABSTRACT*

**Introduction:** High-fat diet consumption results in raised levels of cholesterol in the blood with a massive effect on tongue mucosa, according to previous research. Recently it was claimed that using natural products such as curcumin and chitosan has the potential to decrease cholesterol levels, which positively affects the dorsal surface of the tongue. **Aim:** This work purposed to assist the effects of curcumin and chitosan as a natural product on the dorsal surface of tongue mucosa of albino rats fed on a high-fat diet**. Materials and Methods:** 40 rats were divided into 2 main groups (1 – control group: received normal diet and distilled water, 2 – experimental groups: divided into 3 subgroups: 2-A received High fat diet and Cholesterol powder (10gr), 2-B curcumin treated group at the beginning of the fourth month with 1.5 g curcumin/ kg powder dissolved in distilled water, and 2-C chitosan treated group at the beginning of the fourth month with 500 mg/ Kg. BW Chitosan tab which added to a distilled water in a concentration of 10% (curcumin and chitosan were administered to rats via gastric tube). Cholesterol level in blood was measured at days 0, 90 and 120. Rats were euthanised at the end of 4 months, and tongue specimens were processed for histological and immunohistochemical evaluation. All data were statistically analysed using a one-way ANOVA (F) test with a post hoc test (Tukey). **Results:** A high-fat diet caused degeneration to the tongue's dorsal surface; treatment with curcumin and chitosan showed a better histological effect compared with high fat diet group. Chitosan showed near-normal papillae structure with partial muscle regeneration. **Conclusions:** A high-fat diet induces cytotoxic effects on tongue mucosa, while curcumin and chitosan demonstrate protective potential.

## INTRODUCTION

A high-fat diet means consuming an enormous amount of fat, especially saturated fat. Taking too much saturated fat led to raising the harmful cholesterol levels in the body, which resulted in increasing the chance of producing heart disease and other health-offensive conditions. Furthermore, high-fat diets (HFDs) are frequently associated with an enormous consumption of calories, which can contribute to weight gain and obesity [(1)].

Chronic vulnerability to a high-fat diet might result in cholesterol precipitation in the blood (hypercholesterolaemia). High plasma total cholesterol (TC) levels are associated with coronary heart disease, atherosclerosis, and strokes [2].

Hypercholesterolaemia is a condition specialised by high cholesterol levels that can be managed with a variety of medications, including 3-hydroxy-3-methylglutaryl coenzyme A (HMG-COA) reductase inhibitors, bile acid sequestrants, activated lipoprotein lipase inhibitors, and cholesterol absorption inhibitors [3].

The most common enhancement methods for hypercholesterolaemia are to reduce the conducting of pharmaceuticals such as statins and fibrates, which can have a variety of side effects. As a result, investigating different medicines, such as natural supplements, for managing hypercholesterolaemia has lately become an acceptable plan against hypercholesterolaemia [4].

Most lipid-lowering variables are accompanied with significant treatment problems and serious adverse effects. In contrast, dietary fibres provide a safer option for lipid-lowering medication. Chitosan (CS), a dietary fibre, is biodegradable, biocompatible, and has numerous health benefits. These advantages include wound healing, anti-inflammatory and anti-cancer qualities, immunological modulation, haemostatic effects, lipid-lowering capacities, and antioxidant activity [5].

Chitosan has shown promising effects on oral tissues, particularly in wound healing, antimicrobial activity, and biomimetic mineralization. It enhances the healing process of oral mucosa by recruiting neutrophils and macrophages, stimulating angiogenesis, and promoting tissue regeneration. Additionally, chitosan has been explored as a biomaterial for preventing and treating dental caries due to its antibacterial properties, ability to support mineralization, and potential for drug delivery [6].

Furthermore, chitosan has also been used in wound treatment, particularly in periodontal therapy for tissue reconstruction purposes, as it has minimal body reaction and the potential to promote cell proliferation of human gingival fibroblasts and stimulate wound healing. This biopolymer has been reported to play an important role in the early phase of wound healing because it increases the infiltration of polymorphonuclear cells (PMNs). It stimulates macrophages and mononuclear cells, as well as the synthesis of various growth factors that enhance wound healing and bone formation [7].

Chitosan is not fully digested and absorbed in the body; yet, it has been indicated as a factor in improving weight and cardiovascular risk factors [8].

Curcuma longa, a transmitted medicinal plant, is extensively scattered in China and other Asian nations. Turmeric, the rhizome of Curcuma longa, is consumed as a spice to increase flavour and as medicine because of its medicinal effects [9].

Curcuminoids, the principal bioactive compounds derived from the rhizome of Curcuma longa L., resulted in the major biological consequences of turmeric [10].

Experimental and clinical investigations have discussed the positive effects of curcumin addition on lipid profile and glycaemic condition [11].

Curcumin has demonstrated various therapeutic effects on oral tissues, including anti-inflammatory, antioxidant, and antimicrobial properties. Studies suggest that curcumin can help regulate bacterial plaque, control gingivitis, and support wound healing in conditions like oral mucositis, leukoplakia, and oral lichen planus. Additionally, curcumin has been found to enhance collagen density and cellular regeneration, making it beneficial for oral submucous fibrosis [12].

In **Adhikari's 2022** [13] study, curcumin, known for its anti-inflammatory, antioxidant, and fibrinolytic properties, was shown to have anti-tumour capabilities, reducing the activity of inflammatory and cell growth factors associated with precancerous and cancerous conditions. This polyphenolic compound has demonstrated beneficial properties in the treatment of oral pathologies such as oral submucous fibrosis (OSMF), a chronic and progressive disease that can lead to malignant transformation.

In previous study, the bio-enhanced turmeric formulation (BTF) used consisted of a mixture of curcuminoids, mainly curcumin and turmeric essential oil, showing a significant reduction in severe oral mucositis, dysphagia, oral pain, and dermatitis in patients with oral cancer undergoing chemotherapy. Sixty patients who underwent radical surgery were randomized into three groups: groups A and B received bio-enhanced curcumin capsules at a low dose (1 g/day) or a high dose (1.5 g/day), respectively, and group C received the placebo. The treatment was administered daily for 6 weeks in conjunction with chemoradiotherapy [14].

Statins and fibrates are common pharmacological treatments for hypercholesterolaemia, but they can cause a variety of side effects. As a result, discovering an alternative drug, such as curcumin and chitosan, to manage hypercholesterolaemia has proven to be an effective strategy [15].

## MATERIALS AND METHODS

### Sample Size Calculation

The sample size was calculated using G*Power version 3.19.2, Franze Faul, University Kiel, Germany. Copyright© 1992-2014. By using the equation

$$\frac{Z^2XP(1-P)}{e^2} \div 1+(\frac{z^2XP(1-p)}{e^2n})$$

The effect size is 0.8 using both the alpha (α) and beta (β) levels of 0.05, i.e., power = 95%; the estimated sample size (n) should be at least 40 rats for 4 groups, with 10 rats in each group [16].

### Grouping:

40 adult male albino rats with a mass weight of 160-180 gm each at the initiation of the experiment.

Rats were adjusted 7 days before starting the experiment. Rats were kept under adequate ventilation in separate cages (5 rats each) at the animal house of the Faculty of Dentistry, Suez Canal University. The present research was administered after the acceptance of the Research Ethics Committee (REC) of the Faculty of Dentistry, Suez Canal University, in approval number 420/2021. Rats were divided into two main groups as follows:

### 1- Control group (- ve control):

This group consisted of 10 rats that received a typical diet and distilled water through a gastric tube for 4 months, and they served as a control for other groups.

### 2- Experimental Groups:

Were subdivided into 3 subgroups (n=10):

***2- A High Fat Diet Group (+ve control):***

Rats received 1% cholesterol in their diet for 4 months. A cholesterol-rich diet was prepared by a mixture of cholesterol (10 g), casein (120 g), salt mixture (50 g), vitamin admixture (10 g), soybean oil (250 g), choline (0.4 g), cellulose (130 g), corn starch (429.6 g), and bile salt admixture (2.5 g), essential for intestinal assimilation of cholesterol[17].

***2- B High Fat diet + Curcumin group:***

Rats was fed a high-fat diet for 4 months. Curcumin was delivered via gastric tube at the start of the fourth month in a daily dose of 1.5 mg/kg BW. Dried powder was received from the local market in Cairo (Harraz) for herbs [18].

***2- C High Fat diet + Chitosan group:***

Rats was fed a high-fat diet for 4 months. Chitosan tablets were crushed by mortar and pestle and dispersed in distilled water in a concentration of 10% and delivered by gastric tube at the start of the fourth month in a daily dose of 500 mg/kg BW [19]. Chitosan tablets were obtained from Sigma Aldrich, USA.

**Evaluation Methods:**

***1. Biochemical Analysis:***

Cholesterol level was measured before application of hypercholesterolaemia (day 0), after application of hypercholesterolaemia (day 90) and at the end of the experimental period (day 120) after the rat's treatment with chitosan and curcumin. Blood samples were collected from the orbital sinus of the rat [19].

***2. Histological and immunohistochemical Evaluation***

Rats were euthanised at the end of 4 months. They were euthanised by excessive-dose inhalation of ether. After euthanization, tongue specimens were collected to accommodate histological and immunohistochemical evaluation.

**Samples preparation for H&E examination:**

At the end of four months, each group's tongue was collected, and the samples were immediately fixed in a 10% buffered formalin solution for at least 48 hours. The specimens were adjusted and stained with haematoxylin and eosin [20].

**Samples preparation for immunohistochemical Examination:**

All tongue specimens regained for immunohistochemistry were fixed in freshly prepared 4% (w/v) paraformaldehyde in 0.1 mol PBS $l^{-1}$ and litigated overnight in paraffin wax. Formalin-fixed paraffin-embedded sections were exposed to remove the wax in xylene and rehydrated via graded alcohol to distilled water. The sections were exposed to antigen regaining by boiling in a microwave for 20 min in 0.01 M sodium citrate buffer (pH 6.0). The dominant antibody to caspase-3 (Transduction Laboratories, Lexington, KY) was added at a dilution of 1:1000 and stored overnight at 4°C. After incubation, the slides were managed with biotinylated rabbit and mouse immunoglobulin (1:600 for 30 min; Dako Ltd., Ely, UK), washed as before, and then managed with streptavidin and biotinylated alkaline phosphatase according to the manufacturer's instructions (Dako) [21].

**Statistical Analysis:**

Statistical analysis was assessed by the Statistical Package for the Social Sciences (SPSS) version 28 (IBM Co., Armonk, NY, USA), a commercially available software program for Windows. Quantitative data and numerical data were introduced as the mean, standard deviation (SD) and range, analysed across time points using repeated measures ANOVA, while analysed across different groups using a one-way ANOVA (F) test with a post hoc test (Tukey). A two-tailed P value $<0.05$ was statistically significant. Mean lipid profile level (biochemical analysis) comparison between all groups was statistically examined.

**Java Based Image Immunohistochemical localization of Caspase III:**

Evaluation of colour intensity and stained area % of immunostaining Immunohistochemically stained sections were assessed using the Leica Quin 500 analyser and the Java-based image processing program (Image J) on the computer system of the veterinary faculty at Beni-Suef University. The image analyser was measured automatically to transform the measurement units (pixels) formed by the image analyser program into exact micrometre units. Immunostaining was calibrated as area percent in a standard measuring frame in 15 fields in each group, valuing a magnification (x400) by light microscopy transport to the screen. The areas showing brown diaminobenzidine (DAB) immunostaining were elected for evaluation [22].

## RESULTS

**Histological Results (H&E):** Histological sections of **control group** rats showed the filiform papillae were distributed evenly over the dorsal surface of the anterior 2/3 of the tongue with keratinised stratified squamous epithelial coverage **(Fig. 1A).** Only the fungiform papilla was seen dispersed between the numerous filiform papillae with broader surfaces and highly vascular connective tissue cores **(Fig. 1B).** pure mucous acini of weber salivary gland between the muscles of the tongue **(Fig. 1C).** well-organised muscle bundles **(Fig. 1D). The high-fat diet group** the filiform papillae exhibited atrophic changes, hyperkeratosis, and cytoplasmic vacuolization in epithelial cells **(Fig. 1E).** The fungiform papillae showed epithelial atrophy, degenerated taste buds, and surface keratinization. Basal cells lost their normal architecture, with focal basement membrane disruption and inflammatory infiltration **(Fig. 1F).** The lamina propria displayed collagen fiber degeneration, inflammatory infiltration **(Fig. 1F).** mucous acini of weber salivary gland appeared with marked degenerative changes in the form of cytoplasmic vacuolisation and cystic transformation **(Fig. 1G).** Lingual muscles showed disorganised and degenerated muscle fibres with multiple spaces in between and inflammatory cell infiltrations **(Fig. 1H). Hyperlipidaemic rats treated with curcumin** showed bitter histological picture compare to high fat diet group. Filiform papillae showed partial regaining of their shape, architecture and decrease in epithelial thickness **(Fig. 2A).** underlaying lamina propria exhibited nearly normal connective tissue with muscle fibers running in different directions which regained its arrangement. However, there were multiple vacuoles noticed in between. inflammatory cell infiltrations were also observed **(Fig. 2A).** Fungiform papillae with vacuolated taste buds **(Fig. 2B).** The mucous acinar cells showed cystic transformation of some cells **(Fig. 2C).** Almost organised muscle bundles with some vacuoles **(Fig. 2D). Hyperlipidaemic rats treated with chitosan** showed adequate normal histological picture in comparing with high fat diet group. The filiform and fungiform papillae showed almost normal histological appearance except for some vacuolisation The surface epithelium of papillae almost regains its normal thickness. The lamina propria showed an apparent increase in cellularity and vascularity as well as normal density of collagen fibers **(Fig. 2E, 2F).** mucous acini showed an almost normal histological picture with well-formed acini and ducts **(Fig. 2G).** The muscles of the tongue were partially regenerated with some vacuoles in between muscle fibres. Blood vessels engorged with blood **(Fig. 2H).**

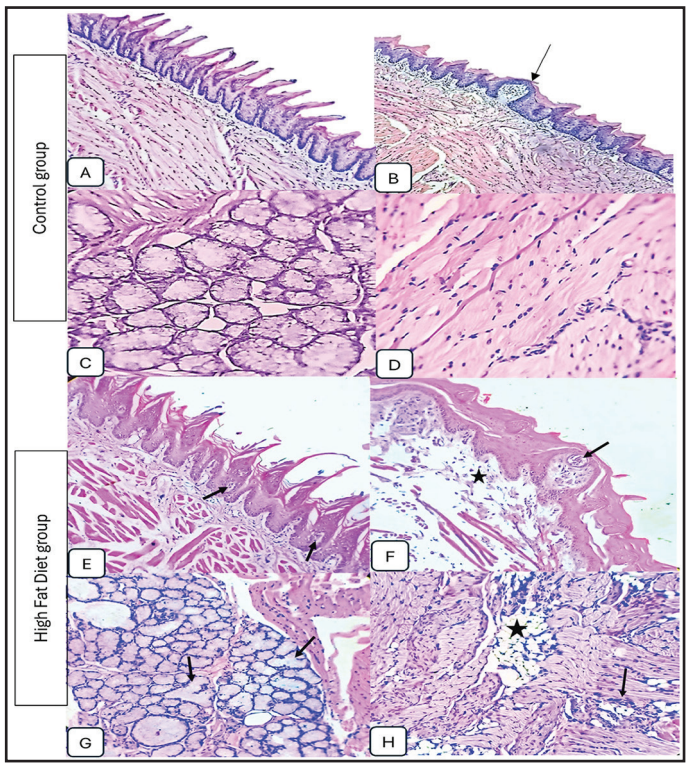


Fig. (1) Photomicrograph showing the control group: **A, B, C, D. High-fat diet group: E, F, G, H. A:** Sharp conical projections of filiform papillae with keratinised stratified squamous epithelial coverage. **B:** a fungiform papilla with connective tissue core (arrow). **C:** Pure mucous acini of Weber salivary glands. **D:** Normal distribution of muscle bundles. **E:** Atrophied filiform papillae with cytoplasmic vacuolisation of epithelium (arrows) and hyperkeratinisation. **F:** fungiform papilla with degenerated taste bud (arrow) and dissociation of collagen fibres (star). **G:** mucous acini with cystic transformation (arrows). **H:** Degenerated muscle fibres with multiple spaces in between (Star) and inflammatory cell infiltrations (Arrow) **(H&E. A, B, E, F, G, H: x160. C, D: x400.).**

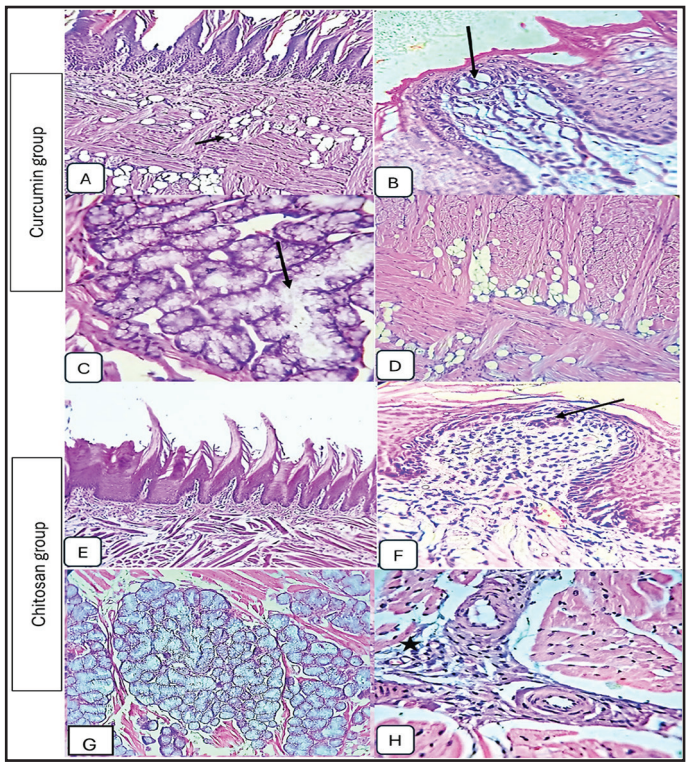


Fig. (2) Photomicrograph showing the curcumin-treated group: **A, B, C, D.** Chitosan Treated group: **E, F, G, H. A:** Filiform papilla with a decrease in epithelial thickness. Underlaying lamina propria with muscle fibers running in different directions which and multiple vacuoles noticed in between (Arrow) **B:** a fungiform papilla with a vacuolated taste bud (arrow). **C:** Mucous acini with cystic transformation (arrow). **D:** Almost organised muscle bundles with some vacuoles (Arrow). **E:** Filiform papillae with nearly normal keratinisation and underlying lamina propria. **F:** Almost normal fungiform papillae with well-organised taste buds (arrow) **G:** mucous acini showing well-formed acini and ducts. **H:** well-organised muscle bundles with blood vessels engorged with blood (Star) **(H&E. C, G: x160). (A, B, D, E, F, H: x400.).**

**II- Immunohistochemical Results:** Immunohistochemical assessment of the surface epithelium of the lingual mucosa and lingual glands revealed the control group was negative to weak for cytoplasmic caspase 3 immunoexpressing in the epithelial cells and basement membranes, with a mean colour intensity percentage of 3.19 ± 13.78% (**Fig. 3**). Rats fed a high-fat diet showed strong cytoplasmic caspase-3 immunoexpressing in the epithelial cells, with a mean colour intensity percentage from 50.66 to 88.25% with a mean of 68.46 ± 11.56%. Rats treated with curcumin revealed moderate immune expression to caspase 3 with a mean colour intensity percentage of 34.23 ± 6.93%. The chitosan-treated group showed weak immune expression to caspase 3 with a mean colour intensity percentage of 13.13 ± 3.13%.

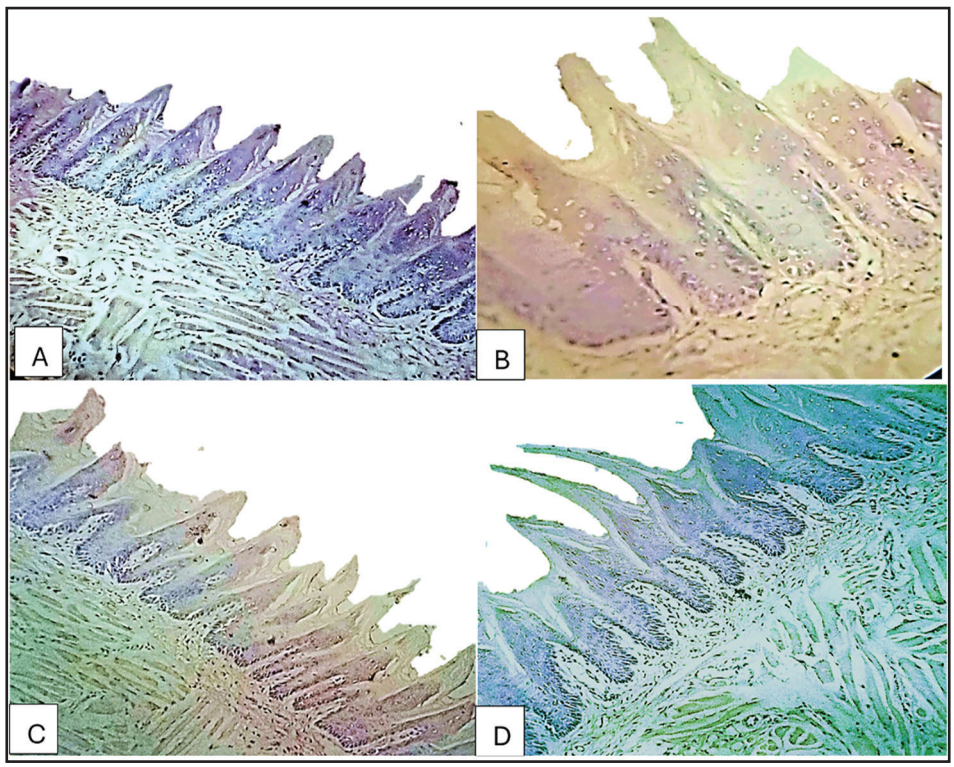


Fig. (3) Photomicrograph showing **A.** Control group, **B.** High-fat diet group, **C.** Curcumin-treated group, **D.** Chitosan- treated group**. A:** weak cytoplasmic and nuclear caspase 3 immunoexpressing in the epithelial cells **B:** strong cytoplasmic and nuclear caspase 3 immunoexpressing in the epithelial cells **C:** moderate cytoplasmic and nuclear caspase-3 immunoexpressing in the epithelial cells. **D:** showing weak cytoplasmic caspase 3 immunoexpressing in the epithelial cells **(Caspase 3A, C, D orig. mag. 160). (Caspase 3B orig. mag. 160**

## III- Statistical results

### Biochemical Analysis

- ***Comparison of blood cholesterol levels among the studied groups***

At day 0, there was not a statistically significant difference among the groups studied in terms of cholesterol level. At day 90, a statistically significant difference was explored among groups (P<0.001), as cholesterol level was significantly increased in all experimental groups assimilated to the control group. On day 120, there was a statistically significant difference among groups (P<0.001) as cholesterol level was significantly lower in the chitosan-treated group than the curcumin-treated group, significantly decreased in both groups compared to the high-fat diet group, and in comparison to the control group, it was significantly at a high level in the high-fat diet and curcumin groups but insignificantly different in the chitosan-treated group. **Table (1A, 1B**), (**Fig. 4A, 4B**).

**Table (1)** *A: Comparison of blood cholesterol levels among the studied groups, B: Colour intensity percentage comparison among the studied groups.*

| A | | Control Group (n=10) | High Fat Diet Group (n=10) | Curcumin Group (n=10) | Chitosan Group (n=10) | P value |
|---|---|---|---|---|---|---|
| **Day 0** | **Mean ± SD** | 65.3 ± 9.8 | 63.4 ± 9.86 | 61.6 ± 10.84 | 65.6 ± 12.53 | 0.828 |
| | **Range** | 55 - 89 | 55 - 88 | 50 - 88 | 54 - 89 | |
| **Day 90** | **Mean ± SD** | 69.3 ± 10.07 [a] | 183.4 ± 11.22 [b] | 181.5 ± 9.57 [b] | 179.3 ± 9.9 [b] | **<0.001*** |
| | **Range** | 55 - 87 | 169 - 198 | 169 - 195 | 169 - 195 | |
| **Day 120** | **Mean ± SD** | 64.2 ± 5.73 [a] | 235 ± 32.91 [b] | 114.2 ± 11.48 [c] | 67.6 ± 9.18 [a] | **<0.001*** |
| | **Range** | 55 - 75 | 195 - 270 | 100 - 130 | 55 - 80 | |

**Colour intensity percentage**

| B | | Control Group (n=10) | High Fat Diet Group (n=10) | Curcumin Group (n=10) | Chitosan Group (n=10) | P value |
|---|---|---|---|---|---|---|
| **Colour intensity percentage** | **Mean ± SD** | 13.78 ± 3.19 | 68.46 ± 11.56 | 34.23 ± 6.93 | 13.13 ± 3.13 | **<0.001*** |
| | **Range** | 10.14 - 19.8 | 50.66 - 88.25 | 25.26 - 45.24 | 10.25 - 19.74 | |
| **Pairwise comparison** | | **P1<0.001***, **P2<0.001***, P3=0.997, **P4<0.001***, **P5<0.001***, **P6<0.001*** | | | | |

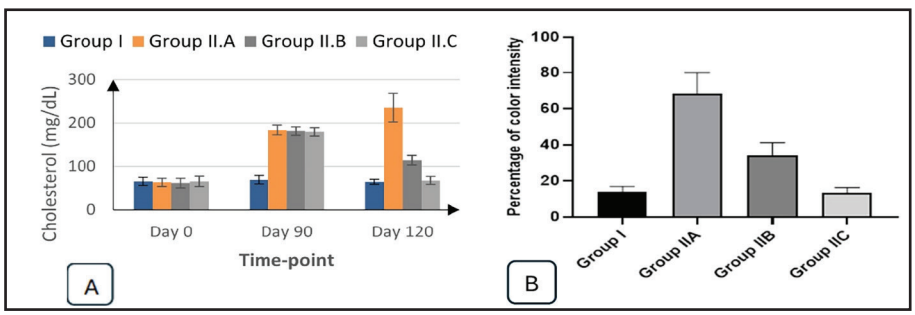


Fig. (4) A histogram showing: A: Comparison between groups Cholesterol blood level. B: Comparison of caspase 3 color intensity percentage among the studied groups

## DISCUSSION

A high-fat diet (HFD) involves consuming a lot of fat, especially saturated fat. Taking too much saturated fat can raise the levels of harmful cholesterol in the body, increasing the chance of resulting in heart disease and other health issues [23].

The use of naturally derived food supplements in health promotion has been fast-growing. Dietary supplements have been demonstrated as an alternate technique for the stoppage and/or treatment of dyslipidaemia and concomitant cardiovascular events, with minimal side effects and toxicity [24].

The study focuses on the dorsal surface of the tongue, as it has four types of lingual papillae. The filiform papillae are more widely distributed, and their cells have a high metabolic activity and easily affected by any disturbance before any other papillae. Tongue manifestations also are linked to diseases which aiding diagnosis and treatment. hypercholesterolemia which induced in albino rats by exposure to diet rich in fats with cholesterol crystals for extended periods (4 months) leads to significant elevation of cholesterol in plasma lipid profile which markedly affect the dorsal surface of the tongue [25,26].

In this study 1.5 mg/kg curcumin was used, as using curcumin is cost-effective nutritional supplement. According to previous research using curcumin in such a dose for treatment of hypercholesterolemia resulted from supplementation of rats with high fat diet showed an enhancement of cholesterol absorption, accumulation, and transport in albino rats, decreasing cholesterol precipitation and atherosclerosis in mice fed high-fat diets. [18,27].

The current study utilised chitosan, a dietary fibre, to control hypolipidaemia and improve hepatic lipid metabolism, potentially resolving hyperlipidaemia due to its higher water solubility. Chitosan was used in a dosage of 500 mg/ kg as this dosage were adequate for treatment of hyperlipidemic massive destruction in 4 weeks of treatment according to previous research [19,28].

So, the present study was designed to compare the effects of curcumin and chitosan (natural products) on the dorsal surface of the tongue mucosa of high-fat-diet albino rats. In the present study, an obvious degenerative histological change of the dorsal tongue mucosa after a high-fat diet was added to albino rats for 4 months. Epithelium of the dorsal surface showed vacuolisation, atrophy, fungal infection and hyperkeratinisation.

Research shows high-fat diets negatively impact the oral cavity, increasing the risk of periodontal diseases, dental caries, and impaired immune function, increasing susceptibility to infection [29].

The destructive effects of a high-fat diet on the dorsal surface of the tongue could be explained according to previous research illustrated that oxidative stress is the main oblation factor in obesity-related diseases. This obesity-related oxidative stress can result in severe cellular damage and dysfunction [30].

Our histological findings showed a filiform mucosal epithelium with flattening papillae and hyperkeratosis, previous study found obesity-related differences in mucosal epithelium, thicker epithelium, higher keratinised granular and prickle layer cells, shorter papillae, and inflammatory cell infiltration which were parallel with our results [31].

The study found hyperkeratosis, an increase in the keratin layer due to inflammation from high fat consumption, which acts as a barrier against fat intake. This aligns with previous research showing high-fat diets can affect the skin's epithelial keratin layer, leading to water loss and increased irritant susceptibility [32].

The high-fat diet group exhibited degenerative changes in lingual glands, including mucous acinar cells, cystic transformations, and inflammatory infiltration, which is consistent with **Pişiriciler** [33] research on lipid aggregation in salivary glands and inflammation. Biochemical analysis revealed a significant increase in cholesterol, like **Munshi et al.'s** [34] findings on albino rats fed different fats.

The study found that curcumin treatment improved regeneration in hypercholesterolemic rats' epithelium of filiform and fungiform papillae, although vacuoles and inflammatory cell infiltration were present. This aligns with previous research suggesting curcumin may lower cholesterol absorption and increase cholesterol-7α-hydroxylase activity, a crucial enzyme in cholesterol catabolism[35].

Curcumin improved lingual glands with multiple cytoplasmic vacuoles, indicating a hypolipidemic effect on rats' serum lipid profile. Curcumin's anti-inflammatory effect reduces keratin layer overlay in filiform papillae, preventing the body from producing more keratin. This effect is attributed to curcumin's cardioprotective susceptibility, which decreases total plasma lipids and peroxidised lipids, highlighting its potential for dietary supplementation. [36,37,38].

The study found that curcumin treatment significantly decreased blood cholesterol levels in rats, confirming previous research that curcumin can lower lipid profiles in rats on a high cholesterol diet. This was attributed to curcumin manipulating hyperlipidaemia by lowering cholesterol and triglyceride levels [39,40].

The study found that chitosan treatment improved the dorsal mucosa of the tongue, with normal appearance of filiform and fungiform papillae. Chitosan could engage more bile acids than cholestyramine, potentially causing a hypolipidaemic effect. Serous and mucous acinar cells showed normal histological appearance, and tongue muscles partially regenerated with inflammatory cell infiltrations and vacuoles. Chitosan's enhancement effect is due to its mechanisms of preventing lipid storage and lipid breakdown [41,42].

Chitosan has been found to enhance the Von Ebner and Weber salivary glands, affecting intraluminal lipid assimilation. Histological findings show normal blood vessels in the chitosan-treated group, indicating its role in promoting blood vessel formation and angiogenesis. Biochemical results show a significant decrease in blood cholesterol levels, and consuming chitosan by HFD-fed rats reverses significant weight gain compared to untreated HFD-fed rats [43,44,45].

The study found that a high-fat diet group showed strong cytoplasmic caspase-3 immunoexpressing in epithelial cells, possibly due to cytokine activation of receptors with 'death domains'. Curcumin-treated rats showed moderate caspase-3 expression, which triggers apoptosis by triggering DNA fragmentation and cell death. This process involves cytochrome C release, complex formation with caspase-9, and caspase-3 activation, leading to phosphatidylserine translocation to the outer plasma membrane. Chitosan weak cytoplasmic expression is explained as in previous research, which showed that application of chitosan on diabetic rats can lower reactive oxygen species (ROS) formation and antioxidant enzyme levels, lowering caspase-3 expression, which plays a primary role in cardiac cell apoptosis [46].

Accordingly, the present study revealed that curcumin and chitosan could introduce alternative treatment for decreasing hypercholesterolemia complications. however, Chitosan representing more promising effect than Curcumin.

## CONCLUSIONS

The research concluded that a high-fat diet has significant cytotoxic effects on the dorsal surface of the tongue. Both curcumin and chitosan may reduce hypercholesterolaemia-induced tongue cytotoxicity, with chitosan showing a better preventive effect than curcumin.

## RECOMMENDATIONS

- Further studies are recommended for combining curcumin and chitosan, using different doses, nanoscale applications, and different administration protocols for hypercholesterolemia prevention.
- Further investigations using transmission electron microscopes and histological structures are also recommended.

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